Participant Information Sheet

Running Blue: Can an acute bout of blueberries boost post-exercise induced benefits to cognitive function?

Study title: ***'Running blue': The effects of blueberry intake on cerebrovascular and cognitive responses to exercise in low and high-fit young adults (ETHICS ID: ERN_19-1574P A8)***

Primary Researcher

Mr Alexander Friend a.t.friend@bham.ac.uk

Investigators

Dr Catarina Rendeiro (Principal) c.rendeiro@bham.ac.uk

Dr Sam Lucas (Co) s.j.e.lucas@bham.ac.uk

Prof Claire Williams (Co) claire.williams@reading.ac.uk

An invitation to take part:

Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study. Before you decide if you want to participate or not, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you wish to take part, or not.

1. What is the purpose of this study?

Decline in 'our ability to think' is part of natural aging and is partly due to deficits in blood flow delivering oxygen and nutrients to the brain. Evidence shows that being physically active improves blood flow to the brain and results in better cognition later in life. We have shown that better cognition can be seen after a single bout of exercise. As such, enhancing exercise-induced changes in brain blood flow has the potential to result in better cognition, and over the longer-term optimizing the benefits of physical activity can be a way to improve resilience against cognitive decline later in life. Critically, there is evidence showing that individuals with low levels of fitness have lower increases in brain blood flow when engaging in moderate/high intensity physical activity, when compared to high fit individuals, and this is also linked to poorer cognition. Therefore, dietary strategies, such as intake of blueberries that are rich in flavonoids, which are small molecules naturally present in cocoa, vegetables, and fruits (e.g., berries, tea, citrus fruits, and apples), prior to exercise may help to optimize the adaptive stimulus to exercise for the poor responders (low-fit), whilst still being able to maximize or accelerate benefits in good responders (high-fit). Indeed, we have previously shown that blueberries can result in immediate increases in blood flow and oxygenation in areas of the brain important for cognition, with such improvements resulting in measurable benefits in cognitive function.

Our key objectives are to investigate whether blueberry intake prior to low, moderate, and high intensity exercise results in better brain blood flow and blood oxygenation, which then leads to better cognitive performance. These data will establish whether blueberries might be effective in

optimizing cognitive health in young healthy adults in the context of physical activity, and this work will be important to inform long-term preventive measures for aging-associated cognitive decline, whilst providing more specific recommendations for those physically active and looking to maximize the health benefits of exercise, as well as more sedentary individuals.

Am I eligible to take part?
We would like to recruit those who:

- Are a healthy male or female
- Are aged 18-40 years old
- Do not smoke and do not consume more than 21 units of alcohol per week
- Have no history of cardiopulmonary, cerebrovascular, musculoskeletal affecting the limbs, respiratory, metabolic, metabolic, liver, inflammatory diseases, or neurological illness
    - This may include but not limited to; blood-clotting disorders, hypertension (BP > 140/90 mmHg), diabetes mellitus, anaemia, asthma (only if you take regular/daily medication or require medication before or after exercise), immune conditions, elevated cholesterol, smokers, or have recently had prolonged bed rest.
- Do not have a known allergy to berries. *Please inform the investigator of any other known allergies.*
- Are not on a weight reducing dietary regiment
- Are not taking any dietary supplements, including fatty acids and vitamins
- Are not taking any long-term medication or have been on antibiotics for the last 3 months
- Do not have an infection at present (e.g., cold)
- Do not have a VO_{2max} between *Female*: >34 and <41 and *Male*: >40 and <50 ml/kg/min

Females only: Female participants will be asked to provide details of their menstrual cycle in medical screening questionnaire. It is crucial that we test female participants in a similar phase of their menstrual cycle or similar pill phase of the combined pill oral contraceptive to minimise the effects of fluctuating sex hormones on blood vessel function. Female participants are provided with the option to opt-out of this question during the questionnaire. Female participants are welcome to talk to a female member of the research team on request. If they do not wish to disclose this information, then they will be excluded from the study and will not complete any further procedures detailed in this document.

2. Do I have to take part?

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form declaring that you are choosing to opt-in to the study. You will also be given the option to opt-in or opt-out of giving consent to be contacted for future studies from the named researchers in this document. After signing the consent form, you are free to withdraw at any time and without giving a reason. You should feel under no pressure to participate and if at any time you are asked questions that you are not comfortable with answering (e.g., those asked in screening or questionnaires) you are free to not disclose this information. Though please do bear in mind that all information collected will be kept strictly confidential. If you choose to not participate or participate but then withdraw from the study it will not affect your relationship with the School of Sport, Exercise and Rehabilitation Sciences, or any of the researchers involved in this study. You may also request your data to be deleted. This will not be possible however after the data is published in a report. Please contact

the primary researcher (AF) should use wish to be withdrawn from the study or have your data deleted.

3. What will happen to me if I agree to take part?

You will be invited to sign a consent form and complete questionnaires relating to your levels of physical activity, general health, and readiness to participate as part of the screening procedure for the study. You are encouraged to ask questions prior to and throughout the study if there is anything you do not understand or feel uncomfortable with. Following provision of informed consent and if the information provided in the screening process does not exclude you from the study, your participation in the study starts and you will be booked in for your visits. If you do not meet our eligibility criteria, you will take no further part in the study.

4. What is required of me if I take part?

You will require you to spend some time reading through this participant information sheet (30 min), attend one video call (1 h), and visit the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham three times (1h, 5 h, and 5 h).

You will be asked to complete a **3-day food diary**, and to **refrain from eating certain foods** (such as fruits, vegetables, coffee, tea, chocolate (a detailed list of what you can and cannot eat will be given to you), **drinking alcohol or caffeinated drinks**, **and vigorous exercise 24 h prior to the 2nd and 3rd visit**. You will be asked to have a **regular night sleep** the day before any laboratory visit. You will be also asked to **be fasted for 12 h** (approx. 8pm for an 8am start) before the morning of visits 2 and 3 and only drink water in this time. You will be provided with a standardised breakfast. You will be required to bring, or arrive in, some **light clothing with short sleeves to exercise in** during the visit to the laboratory. You are advised to bring some form of entertainment with you on the laboratory visit, such as a book or laptop with headphones.

5. What do I have to do during each visit?

<u>Participant Information Sheet (10 min)</u>
You will be given the opportunity to read the participant information sheet detailing the experimental protocol(s). Please take your time and read the following information carefully and it is important you understand the specific nature of the research and what will be required of you in this study. You are encouraged to contact the primary researcher (AF) for any further information should you require it.

<u>Study Briefing Video Call (15 min)</u>
This one-hour video call will guide you through the experimental protocol(s) and provide you with the opportunity to ask any questions you may have. We will ask you to sign a consent form stating that you are happy to participate in the study, and have read and understood this information sheet, and had the opportunity to ask questions. We will then ask you to complete some questionnaires about your lifestyle, health, and experiences. You will be then invited to the laboratory at the School of Sport, Exercise and Rehabilitation Sciences.

<u>Laboratory visits (max 11 h)</u>
All laboratory visits will take place at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. The total time demand will be approximately 12 hours over a one-month period and involve three separate visits. There will be a preliminary visit to determine your fitness level, followed by two main trial visits (each separated by 2-4 weeks). The preliminary visit will involve an incremental exercise test to exhaustion to determine your aerobic fitness (1 h). The

remaining two visits will last around 5 h each and involve two repeats of the following procedures 1) resting physiological measurements, 2) incremental intensity cycling, and 3) a cognitive battery of tasks. In between the two repeats of the procedures, you will have a short period of rest while you will be instructed to consume a beverage containing the blueberry powder or a matched placebo.

Visit 1 (Determining your fitness level)
When you arrive for your first visit, we will give you another opportunity to ask any questions about the study. We will then reconfirm your consent to take part in the study and ask you to complete some questionnaires. The research team will first measure your height, body weight. You will have your blood pressure and resting pulse measured; this will involve wearing an inflatable cuff around your arm. Following these health checks, you will then complete an increment cycling exercise until exhaustion. You will begin with a short warm-up and opportunity to complete some stretching. Thereafter, the test will start very easy and build up slowly until you cannot maintain the prescribed workload. It is essential that you complete this exercise test until exhaustion, as it allows us to determine how hard you will cycle for the following visits. If you VO_{2max} value is below <32 ml/kg/min (Female) or <40 ml/kg/min (Male), or above >40 ml/kg/min (Female) or >50 ml/kg/min (Male), then you will be then booked in for the 2nd and 3rd visits. If your VO_{2max} value is between these cut-offs, then you will be excluded to participate in the 2nd and 3rd visits.

Visits 2 and 3 (Experimental Trials)
You will arrive at the laboratory for these two visits in a fasted state. Upon arrival you will complete a 24 h Food Recall Questionnaire to ensure that you complied with the dietary requirements prior session 2 and 3. Afterwards, you will be asked to lie down in supine position 10 minutes before a forearm blood flow functional assessment, that will involve a small cuff around your wrist that will be inflated for 5 mins before being rapidly deflated. The blood flow in an artery in your forearm will be measured before, during, and after this procedure. After this, you will then consume a light breakfast. You will then be fitted with equipment to measure your heart rate, blood pressure, breathing, cranial blood flow, neck blood flow, and forehead oxygenation. Resting measurements will be taken for 15 min before you will complete a three-staged exercise trial. This exercise trial will begin with a 5-min warm-up before undertaking a sequential three-staged exercise trial at 30%, 60%, and 80% of your maximal aerobic capacity (which was determined in visit 1). Each stage will last 8 minutes. During the final five minutes of the 60% and 80% stage, you will be instructed to complete a cognitive task on a screen positioned on the bike. After the three-staged exercise trial is completed, you will be offered a fixed amount of water and given a period of rest. You will then be instructed to complete two post-exercise cognitive tasks. Once the tasks are completed, you will be asked to sit and then asked to consume (within 10 minutes) either a high-flavanol blueberry beverage or the low-flavanol placebo beverage and have a rest period whilst you complete some online questionnaires. These drinks are vegan and contain artificial flavourings and artificial colourings. Following this rest period you will complete another forearm blood flow functional assessment, the three-staged exercise trial with cognitive tasks, and post-exercise cognitive tasks. On completion of all the repeat procedures, we will remove the equipment and you will be free to leave the laboratory.

Estimated timings of protocol are as follows;

Fasting begins	20:00 (day prior)
Arrival at laboratory	08:00
Experimental procedures 1	08:30 – 10:30
Supplement and Rest	10:30 – 11:00

Experimental procedures 2	11:00 – 12:30
Leave laboratory	13:00

Details of measures obtained

- **Heart rate:** This will be monitored using a band fitted around your chest.
- **Blood pressure:** This will be monitored via an automated stress-testing blood pressure monitor using a cuff placed around your upper arm or by attaching a small finger cuff around your ring finger. This cuff will pulsate at regular intervals but should not be uncomfortable.
- **Breathing:** You will be fitted with a mouthpiece that does not restrict your breathing but measures the composition of your expired air and your rate of breathing.
- **Forearm blood flow functional assessment:** During the cuff inflation you will feel a tingling sensation ('pins and needles') or numbness from the pressure of the inflated cuff on your arm and thigh. This will stop once the cuff is deflated. It is very important that you remain as still as possible, so the ultrasound probe does not move during the procedure.
- **Cranial artery blood flow (Transcranial Doppler Ultrasound):** This will require you to wear a cushioned headband and remain still whilst the sonographer positions two small non-invasive probes to each side of your head (slightly in front of your ears) and fix them on the headband.
- **Neck artery blood flow (Duplex Ultrasound):** A trained male sonographer will hold a small non-invasive scanning probe to your neck so they can image blood vessels in your neck. There will be some gel on the probe which might feel cold against your skin, and you will feel the probe pressing gently on your neck, but it should not cause you discomfort
- **Forehead blood oxygenation (Near-Infrared Spectroscopy):** This will be monitored using two adhesive non-invasive probes will be attached to your forehead.
- **Perceived exertion/ability to cope with cycling:** These will be measured using charts shown to you prior to starting and periodically during the trials.
- **Questionnaires:** Short questionnaires evaluating physical activity levels and diet.

6. What are the possible disadvantages and risks of taking part?

We encourage you to take your time to have a read of this participant information sheet and have a think whether you are comfortable to take part in this study.

Exercise Testing*:* Performing moderate-to-vigorous exercise carries the following risks:

- Sensations of fatigue and physical exhaustion – this will be short lived and will subside shortly after exercise is ceased. You are free to stop exercising at any time during the incremental exercise trial.
- Fainting – often related to physical exhaustion and sudden stopping of exercise. The inclusion of a warm-down will mitigate this risk.
- Cardiovascular event (e.g. myocardial infarction or 'heart attack') – This is a very small risk, particularly for healthy individuals. We will also ensure that you are warmed up and cooled down appropriately around the exercise sessions and will be monitoring your heart rate and general disposition when exercising to minimise the risk of this happening.

Trained investigators will supervise the exercise sessions, and there will be at least one CPR-certified investigator with automated external defibrillator (AED) training present during the visit. If at any time during the test you want to stop, you can signal as instructed and the test will be stopped. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above.

Blueberry powder ingestion*:* Risk of adverse reaction to ingestion of blueberry powder. There are no known risks associated with taking blueberry drinks provided within this study, this are produced by a food company for human consumption. The blueberry drinks do not contain any known allergenic compounds (detailed ingredient composition of the blueberry powders can be provided if you wish). The investigators are experienced in performing all the procedures detailed with hundreds of similar sessions completed safely in the recent past. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above

Blood Pressure: The application and inflation of the blood pressure cuff on index finger for long durations may cause some discomfort to you, but there will regular periods where the cuff will be deflated, and it will not be in one location for any longer than is necessary for data collection.

Respiratory: Risk of upper respiratory tract infection. All inspiratory equipment will be sterilized before the study.

Cranium and neck blood flow (Transcranial Doppler and Duplex Ultrasound): Doppler ultrasound are non-invasive and painless procedures, with no adverse effects have been reported to date. Ultrasound waves have the potential to slightly heat tissue and, in some cases, produce small pockets of gas in body fluids or tissue (<0.1%). Moreover, positioning of the Doppler probe at the neck and fixing of the headband to the scalp for long durations may cause some discomfort to the participant (<0.1%). The researchers will employ the 'As Low as Reasonably Practicable' (ALARP) principle when scanning. Exposure time will be minimized, unnecessary scans will not performed, the probe will not fixed in one location for any longer than is necessary, and the initial power settings will be low and the output is increased until a suitable image is observed (thus minimizing the power to the lowest possible to achieve the objective).

Forehead blood oxygenation (Near-Infrared Spectroscopy)*:* NIRS is a non-invasive and painless imaging technique with which to assess tissue haemodynamics and that are no known adverse effects. The source and detector probes are attached to the scalp with adhesive pads which can be removed quickly in case of emergency (such as a fire alarm and evacuation).

What if there is something wrong with my body, would it show up on my assessments?
This is an important question, and one that can't be answered with a straight yes or no answer. The information below hopes to provide an answer. If you still have questions, please ask the researcher for more information.
There is the potential that an incidental finding (IF) will be found in one of the physiological assessments. The likelihood of an IF being identifiable in a healthy volunteer's assessments, so you should be aware that such a possibility exists. The very large majority of such IFs (99% in our experience) are not of clinical relevance, being either benign or normal variants. The physiological assessments are completed as part of the study you are participating in are designed to answer research questions and not to provide a medical diagnosis. They may not show problems that an ordinary clinical assessment would, and since the scientists reviewing the assessments are generally not medical doctors, they may fail to notice such abnormalities. However, if something out of the ordinary is suspected in one of your assessments, the primary researcher will review the physiological assessment with the supervisory team. If supervisory team suggests it, the primary researcher will contact you and offer the opportunity to meet and discuss the findings and decide how to proceed. If you agree that a medical follow-up is appropriate, the primary researcher and supervisory team will provide you with a letter for your GP and a copy of the findings. It will be

your responsibility to give the letter to your GP. If you decide to share the letter or findings with your GP or any other health professional, they may become part of your medical record.
There is the possibility that you may be unduly worried if a problem is suspected but is not actually found. If in the future symptoms do arise, do not assume that because you have completed several physiological assessments and we haven't contacted you that there is not a problem. Please address any future concerns with your GP.

7. What are the possible benefits of taking part?

We will provide you with up to £100 compensation and you can receive up to 11 hours credit for research participation. If you withdraw after the first visit (VO_{2max} test), you will receive no reimbursement. If you withdraw after the second visit, you will receive £30 reimbursement. After completion of the third and final visit, you will receive a further £70 (£100 total) reimbursement. At your request, you will find out about your blood pressure, vascular function (e.g., FMD is a tool used at hospitals to estimate your cardiovascular health), and resting heart rate. You will have the opportunity to take part in a study that uses world class equipment and facilities whilst improving our knowledge of cerebrovascular responses to nutritional supplementation during exercise.

8. Will my taking part in this study be kept confidential?

Yes, your participation in this study will be kept confidential. All data, where you are identifiable (consent forms and medical screening questionnaire), will be managed in accordance with the University's Code of research Practice and the terms and conditions of the Data Protection Act 2018. As part of our standard procedures, you are required to provide your name, age, and contact email for the purpose of giving consent and to liaise with you throughout the study, with all correspondence conducted through a password protected university account. All information collected during online questionnaires will be stored on a password-protected account only accessible by the primary researcher (AF). All information collected as physical copies will be stored in a key only accessible office, in an alarmed building and access to the room will be available by the primary researchers.

From the point of completion of informed consent, your data will be pseudo-anonymised via a coding method to ensure that personal identification is only possible by the research team. Specifically, you will be assigned a unique identification number from a random number generator during the initial screening and this number will be used thereafter. The key to identifying your name to your assigned code will be stored, on a password encrypted electronic file accessible by the primary researcher (AF) on secure University computer. Any online cloud storage will take place from a password-protected account and stored on servers that are located within the European Economic Area (EEA).

All further data collected from you, including non-identifiable personal information such as anthropometric information (height, weight), medical information, and experimental data will be collected using the unique identification number. You will sign an informed consent form before participation, indicating you are happy for any data collected to be used as part of an undergraduate and postgraduate thesis and any associated publications. Should any research findings be published, no information will be disclosed to identify you. All anonymised coded data we collect from you will added to a data repository for purpose of publication. No information will be passed on to any third party for data gathering procedures, or advertisement. In accordance with the University of Birmingham's data storage policy, all data will be stored securely for a minimum of 10 years. After this time, it will be permanently destroyed, provided it is of no further

use to the academic world (if kept, it will remain stored securely and confidentially). If you wish to withdraw from the study, any data already collected with consent, where they cannot be identified, will be retained and used for scientific study. Any data or personal information collected where they can be identified, will be deleted on request.

9. What will happen to the results of the research study?

The results of this project may be published anonymously in a scientific journal and within a postgraduate research thesis; however, names of participants will never be published.

10. Who is organising and funding the research?

The project is externally funded by US Highbush Blueberry Council. Mr. Alexander Friend is conducting the research under the management of Dr. Catarina Rendeiro, Dr. Sam Lucas, and Prof. Claire Williams. This project is part of the Programme of Work entitled: Understanding and optimising how exercise influences vascular health (Ethical Review Number: ERN_19-1574P7).

11. Can I obtain feedback from the study?

Yes, if you wish to know the results of the study you took part in a summary of the results can be provided once the study has concluded. On the Consent Form there is a space to indicate if you would like to receive a study summary.

12. What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis. Additionally, you can withdraw your data from the study for up to two weeks following completion of the data collection, by notifying us via email or telephone. If you withdraw and do not consent to having the data collected so far included in the analysis, the data will be permanently deleted/destroyed.

13. Do you have any further questions?

Thank you for your time reading this document. If there is anything that is not clear, you would like more information or wish to take part in this study, please do not hesitate to contact: